# 加州鲈白云病的病原及血液病理的初步研究

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**摘要**:从患白云病的濒死加州鲈体内分离到一株细菌 01314-1,经人工感染试验及 API20NE 系统鉴定,认为该病原菌为洋葱霍尔德氏菌(*Burkholcepacid*)。血液生化指标检测显示,与健康鱼相比,患病鱼的血糖、总蛋白、尿素、肌酐、胆固醇、$K^+$、$Na^+$、$Cl^-$ 等指标以及谷丙转氨酶 ALT、谷草转氨酶 AST、乳酸脱氢酶 LDH 等血清酶类发生显著改变,说明经洋葱霍尔德氏菌感染后,加州鲈肝、肾、肌肉等重要组织已发生病变,机体正常生理功能严重失调。药敏实验结果表明:复方新诺明、丁胺卡那霉素、磺胺甲基异噁唑、妥布霉素、林可霉素等 5 种化学疗剂及五倍子、乌梅、五味子等 3 种中草药对该病原菌有较强的抑菌作用。

**关键词**:加州鲈;白云病;洋葱霍尔德氏菌;血液生化指标;药敏试验
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加州鲈学名大口黑鲈(*Micropterus salmoides*),属鲈形目太阳鱼科,原产北美,自 20 世纪 80 年代引进国内后,由于经济效益良好,发展很快,现已成为淡水名特优养殖品种之一。但随着养殖规模的扩大,集约化程度的不断提高,疾病也频繁发生。特别是近几年在浙江省的奉化、宁海等地的水库网箱养殖场多次发生加州鲈大规模死亡现象,损失极为惨重。因病鱼症状与国内外报道的鲤鱼白云病症状[1,2]类似,故称加州鲈白云病。为了尽快搞清此病发生的原因,找到有效的防治方法,作者以典型症状的病鱼作为材料,对其病原、血液生化指标及防治药物进行了研究,结果报告如下。

## 1 材料与方法

**1.1 材料** 病鱼和健康鱼均取自浙江省奉化市亭下湖水库养殖场,鱼体重 250—500g。

**1.2 病原菌分离** 取发病症状典型的濒死病鱼,现场用 70%的酒精棉球反复擦拭消毒病鱼的腹部和体表,用无菌剪刀打开腹腔,无菌操作取病鱼血液、肝、肾、肠等组织小块,划线接种于普通营养琼脂培养基上,25 ℃培养 24h 后,挑取形态特征一致的优势菌落进行纯培养,转接斜面保存备用。

**1.3 人工感染试验** 在 60×50×40cm³ 水体的玻璃缸中,每缸放养体重 400—500g 的健康加州鲈 10 尾,连续充气,暂养 1d 后进行腹腔注射、创伤浸泡和浸浴感染试验。试验菌株在 25 ℃下培养 18—24h,用无菌生理盐水制成菌悬液,菌悬液细菌量按 McF 浊度管结合活菌计数方法确定。

**1.4 腹腔注射感染** 将培养菌以无菌生理盐水制成浓度约为 $2.31\times10^8$Cells/mL 的菌悬液,以腹腔注射(0.3mL/尾)的方法进行人工感染试验。对照组注射同量无菌生理盐水。

**1.5 创伤浸泡感染** 以无菌针头对试验鱼体表进行穿刺、刮鳞创伤,创伤总面积约 2—3cm²,使创伤后的鱼在含菌量约为 $2.31\times10^5$ Cells/mL 的暴气自来水中浸泡暂养 24h,换水暂养观察鱼体情况。对照组不加菌液。

**1.6 浸浴感染** 选择体表无损伤的健康加州鲈,放入含菌量约为 $2.31\times10^5$Cells/mL 的暴气自来水中暂养 24h,换水暂养观察鱼体情况。对照组不加菌液。

**1.7 病原菌鉴定** 纯培养的细菌经 25 ℃培养 18—24h 后,作革兰氏染色及鞭毛染色,进行细菌形态观察,生理生化特性应用法国生物梅里埃(biomerieux)公司的 API20NE 细菌鉴定系统和编码手册进行,并参照有关资料[3,4]进行其他相关指标测定。

**1.8 血液生化指标测定** 用 5mL 针筒尾静脉取血,全血制备血清,于 4 ℃下放置 2h,经 5000r/min 离心

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分离,收集血清,当天测定完成。所有生化指标均在 DADE BEHRING 生化自动分析仪上完成。

**1.9　药敏试验**　以涂布法接种病原菌于平板培养基上,贴上药敏纸片(直径 6mm),25 ℃培养 24h 后测抑菌圈直径。所用化学疗剂药敏纸片购自杭州天和微生物试剂有限公司,中草药药敏纸片自制[5]。

## 2　结果

### 2.1　病鱼症状及流行情况

病鱼发病初期体色变深、离群、上浮、活力减退、反应迟钝。随着病情发展,体表分泌大量粘液,形成白色浆状膜,尤其在背鳍两侧及鳃盖附近粘液浓厚,肉眼观察似层层云朵,故名白云病。到了后期,病鱼胸鳍、尾鳍基部严重充血、溃疡,部分病鱼鳞片脱落严重,有的体表多处寄生水霉。解剖观察,内部器官病变明显,胃肠略充血,肝表面有土黄色浊斑,部分呈糜状,肾略肿。一般体表出现症状后,在 1—7d 内死亡。

此病发生在冬末春初,水温 18 ℃以下,水库网箱养殖的加州鲈中,总感染率为 80 %,累计死亡率为 50 %—70 %。池塘养殖的加州鲈中未见此病,如果把发病的加州鲈转养到池塘中或在投喂的饵料中加维生素 C,此病可缓慢好转。

### 2.2　病原菌的致病性

从患病加州鲈体内分离出的优势菌株中挑选 01314-1 作为代表进行人工感染试验,结果见表 1。致病性试验结果表明,01314-1 菌株具有强毒性,对健康鱼的感染试验的致死率均为 100 %,对照均无死亡。健康鱼经过人工感染后出现的症状与自然发病鱼症状相似。在濒死鱼的体表粘液及血液、肝、肾等组织中均可分离到与原病原菌特征一致的菌株,说明 01314-1 菌株确是加州鲈白云病的致病菌。

### 2.3　病原菌的鉴定

实验结果表明,01314-1 菌株为革兰氏阴性短杆菌,菌体直,大小为 0.6—0.9 ×1.6—3.4μm,单个或成双,以极生丛毛运动,无荚膜,无芽孢,在普通营养琼脂培养基上,25 ℃培养 24h,菌落直径为 1—2mm,呈圆形、黄白色、湿润、中间略突起、边缘光滑。该菌不发酵葡萄糖,API20NE 鉴定结果见表 2,查阅编码手册以及相关资料,可知 01314-1 菌株为洋葱霍尔德氏菌(*Burkhol cepacid*),且属于伯克霍尔德氏菌属(*Burkholderid*)。

**表 1　人工感染试验结果**

Tab.1　Results of artificial infection test

| 感染方式 Infected way | 细菌数量 (Cells/mL) Bacteria numbers | 试验尾数 Ind. test nos. | 死亡尾数 Dead nos. | | | | | | | | | | | | 死亡率(%) Mortality |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12(d) | |
| 腹腔注射 Abdominal cavity | $2.31\times10^{8}$ | 10 | 1 | 4 | 2 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 100 |
| 创伤浸泡 Wound | $2.31\times10^{5}$ | 10 | 1 | 0 | 0 | 1 | 1 | 0 | 2 | 1 | 3 | 1 | 0 | 0 | 100 |
| 浸浴感染 Soaking | $2.31\times10^{5}$ | 10 | 0 | 0 | 1 | 0 | 2 | 0 | 1 | 2 | 2 | 1 | 0 | 1 | 100 |

**表 2　01314-1 菌株 API 20NE 系统鉴定结果**

Tab.2　The identity results of 01314-1 strains by API 20NE

| 项目 Item | $NO_3$ | TRP | GLU | ADH | URE | ESC | GEL | PNG | GLU | ARA | MNE | MAN | NAG | MAL | GNT | CAP | ADI | MLT | CIT | PAC | OX |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 试验菌反应 Reaction | + | - | - | - | - | + | - | + | + | + | + | + | + | - | + | + | - | + | + | + | + |
| 数值 Number | 1 | 2 | 4 | 1 | 2 | 4 | 1 | 2 | 4 | 1 | 2 | 4 | 1 | 2 | 4 | 1 | 2 | 4 | 1 | 2 | 4 |
| 被试菌编码 Coding | | 1 | | | 4 | | | 6 | | | 7 | | | 5 | | | 5 | | | 7 | |
| 查码 Check yard | 1467557 | | | | | 洋葱伯克霍尔德菌 *B. cepacid* (洋葱假单胞菌 *P. cepacid*) | | | | | | | | | | | 99.9 % | | | | |

注:+ 阳性,Positive;- 阴性,Negative

### 2.4　患病鲈鱼血液生化指标的变化

健康鱼和患病鱼的血液生化指标值见表 3。与健康鱼相比,病鱼的 11 项主要血液生化指标值均呈现明显的变化。

### 2.5　药敏试验

用纸片法对 01314-1 菌株进行药物敏感性试验,结果见表 4。在所试的 32 种药物中,复方新诺明、丁胺卡那霉素、磺胺甲基异噁唑、妥布霉素、林可霉素等

5 种化学疗剂及五倍子、乌梅、五味子等 3 种中草药对 01314-1 菌株具有较强的抑菌作用。

**表 3 健康鱼与患病鱼的血液生化指标的比较**(n = 10)

Tab. 3 The comparisons of the blood biochemical indices between healthy and diseased fishes (n = 10)

| 项 目 Item | 健康鱼 Normal fish | 病鱼 Diseased fish | *P* |
|---|---|---|---|
| 谷丙转氨酶 ALT(μ/L) Glutamic pyruvic transaminase(μ/L) | 43.00 ±8.14 | 202.00 ±17.57 | < 0.001 |
| 谷草转氨酶 AST(μ/L) Glutamic oxalacetic transaminase(μ/L) | 279.00 ±67.36 | 574.32 ±79.61 | < 0.001 |
| 乳酸脱氢酶 LDH(μ/L) Lactic dehydrogenase(μ/L) | 502.00 ±91.16 | 1341.00 ±118.85 | < 0.001 |
| 总蛋白 TP(g/L) Total protein(g/L) | 41.00 ±4.62 | 62.90 ±5.23 | < 0.001 |
| 尿素 BUN(μmol/L) Urea nitrogen(μmol/L) | 2.62 ±0.41 | 3.54 ±0.81 | < 0.05 |
| 肌酐 Cr(μmol/L) | 42.60 ±1.10 | 47.90 ±1.64 | < 0.001 |
| 总肝固醇 CHO(mmol/L) | 44.46 ±9.18 | 33.70 ±5.36 | < 0.05 |
| 血糖 GLU(mmol/L) | 19.39 ±3.87 | 1.05 ±0.88 | < 0.001 |
| 钾 $K^+$(mmol/L) | 1.90 ±0.45 | 6.50 ±1.36 | < 0.001 |
| 钠 $Na^+$(mmol/L) | 147.13 ±4.67 | 104.00 ±9.15 | < 0.001 |
| 氯 $Cl^-$(mmol/L) | 92.50 ±2.50 | 54.00 ±4.72 | < 0.001 |

## 3 讨论

白云病是国内外鲤养殖中危害较大的鱼病之一。该病最早发现于 1973 年日本长野县流水养鲤越冬池中,从 20 世纪 80 年代中期开始国内的北京、四川、天津、宁夏等多个地区相继报道该病在鲤、鲫养殖中发生,但国内外至今未发现其他淡水养殖鱼类中有类似病例的产生。而近几年在浙江省奉化、宁海等地的加州鲈水库网箱养殖中大规模发生的疾病,无论是病症还是流行情况,都与鲤白云病[1,2]极为相似,因此称该病为加州鲈白云病。

据报道[1,2]日本分离到的鲤白云病的病原菌为荧光假单胞菌 (*Pseudomonas fluorescens*) 的变种,国内分离到的鲤白云病的病原菌为恶臭假单胞菌(*P. putida*),而本研究通过对典型患病加州鲈进行的病原分离、鉴定及人工感染试验,认为近几年浙江发生的加州鲈白云病的病原为洋葱霍尔德氏菌(*Burkholcepacid*),归属于伯克霍尔德氏菌属(*Burkholderid*)[3],该属是 1993 年 Yabuuchi 等人提出的新属,它是从原来的假单胞菌属中分离出来的。在《伯杰系统手册》[4]中,洋葱霍尔德氏菌(*B. cepacid*)称为洋葱假单胞菌(*P. cepacid*),归属于假单胞菌属(*Pseudomonas*)。

洋葱霍尔德氏菌广泛分布于水、土壤等环境中,

**表 4 药敏实验结果**

Tab. 4 The results of drug sensitivity test

| 化学疗剂 Chemotherapelltants | 含药量(μg 或 IU/片) Drug contents | 抑菌圈(mm) Inhibitory rings | 中草药 Chinese herbal medidines | 抑菌圈(mm) Inhibitory rings |
|---|---|---|---|---|
| 强力霉素 Doxycycline | 30 | 12 | 威灵仙 Clematis | 11 |
| 复方新诺明 Compound sulfamethoxazole | 25 | 23 | 连翘 Forsythia | 12 |
| 丁胺卡那霉素 Amikacin | 30 | 26 | 白芍 Paeonia | 13 |
| 四环素 Tetracycline | 30 | 17 | 黄芪 Astragalus | 12 |
| 磺胺甲基异噁唑 Sulfamethoxazole | 10 | 30 | 五味子 Schisandra | 25 |
| 克林霉素 Clindamycin | 2 | 15 | 石榴皮 Punica | 12 |
| 庆大霉素 Gentamicin | 10 | 17 | 大青叶 Isatis | 12 |
| 麦迪霉素 Midecamycin | 30 | 0 | 地榆 Sanguisorba | 15 |
| 妥布霉素 Tobramycin | 10 | 28 | 地丁 Corydalis | 10 |
| 氨苄青霉素 Ampicillin | 10 | 19 | 公丁香 Eugenia | 15 |
| 头孢孟多 Cefamandole | 30 | 14 | 木瓜 Chaenomeles | 14 |
| 卡那霉素 Kanamycin | 30 | 15 | 乌梅 Armeniaca | 31 |
| 氟哌酸 Norfloxacin | 10 | 19 | 黄岑 Scutellaria | 14 |

续表

| 化学疗剂 Chemotherapelltants | 含药量(μg 或 IU/片) Drug contents | 抑菌圈(mm) Inhibitory rings | 中草药 Chinese herbal medidines | 抑菌圈(mm) Inhibitory rings |
|---|---|---|---|---|
| 林可霉素 Lincomycin | 2 | 29 | 大黄 Rheum | 14 |
| 链霉素 Streptomycin | 10 | 15 | 板兰根 Isatis | 15 |
| 新生霉素 Novobiocin | 5 | 16 | 五倍子 Rhus | 24 |

它可引起人和动植物的多种疾病,如败血症、伤口感染、脓肿等[6,7]。人工感染结果显示,洋葱霍尔德氏菌对加州鲈具有极强的致病性,病鱼症状与自然发病鱼相似。与健康鱼相比,患病鱼在多项血液生化指标上都发生了显著的变化。ALT、AST、LDH 等血清酶类主要分布于机体的肝、肾、肌肉等组织细胞中,在正常情况下血清中这些酶活性低且相对恒定,而病鱼中 ALT、AST、LDH 等酶显著上升,血糖、胆固醇等下降,说明病鱼的肝、肾、肌肉等组织,尤其是肝脏组织已发生严重损坏,细胞内酶大量逸出,机体糖、脂肪、蛋白质等物质代谢发生障碍。血清中的尿素、肌酐、$K^+$、$Na^+$、$Cl^-$ 等的含量是肾功能的指标[8—10],它们发生了显著变化,说明鱼体肾单位滤过及重吸收功能失调,肾功能不全。血清总蛋白、尿素、肌酐等的上升,显示鱼体严重脱水、血液浓缩[9],这与病鱼分泌大量粘液的现象也相符合。因此,作者推测洋葱霍尔德氏菌感染鱼体后,可能引起鱼体肝、肾、肌肉等重要组织严重病变,使鱼体各项正常的生理功能失调,病鱼分泌大量粘液,造成机体严重脱水,最后休克死亡。而关于洋葱霍尔德氏菌具体的致病机理还有待于进一步研究。

药敏试验结果表明,复方新诺明、丁胺卡那霉素、磺胺甲基异噁唑、妥布霉素、林可霉素、五倍子、乌梅、五味子等药物对 01314-1 病原菌有较强的抑菌作用,可适当用作预防和治疗加州鲈白云病的药物。

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# PRELIMINARY STUDIES ON PATHOGEN AND HEMOPATHOLOGY OF THE WHITE CLOUD DISEASE IN MICROPTERUS SALMOIDES

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**Abstract:** *Micropterus salmoides* was one of the chief fresh water rearing breed in China. As a result of rearing scale enlarging and intensive degree improving, the disease occurred frequently. Especially in the lately year large-scale *Micropterus salmoides* died repeatedly in the reservoir cage culture in Fenghua and Ninghai of Zhejiang province, which caused a large loss for the breeding. Much slime around the body of fish was an important sign of the diseased fish. At the beginning of disease, the fish body color started to become deep, and exercise ability decreased, and float on the surface of the water. When the disease became systemic, fin would be bloody and ulcer, and the scales of some diseased fish dropped seriously, and some fish body had parasitic *saprolegnia*. The gut would be diseased, the stomach and intestines would be bloody, liver would be yellow spot. The fishes would be dead in seven days after the symptom began. Because the symptom of diseased fish was similar to that of the white cloud disease of carp, this disease was called the white cloud of *Micropterus salmoides*. The white cloud disease was one of the best harmful disease in carp rearing in the world. This disease was firstly found in 1973 in Japan. At 1980's, this disease was reported in carp rearing in some areas of China, but this disease was firstly found in *Micropterus salmoides* rearing. The disease occurred between winter to spring, with the water temperature below 18 ℃. In the reservoir cage culture of *Micropterus salmoides*, the total infection rate was 80 % and mortality was from 50 % to 70 %. *Micropterus salmoides* in the ponds had never been found the white cloud disease. If the diseased fish was placed in the pond and was eaten by bait adding vitamin C, the diseased fish would recover.

Diseased fish and health fish came from the rearing reservoir in Fenghua of Zhejiang province, and the fishes weighed from 250g to 500g in the study.

One strain of bacteria 01314-1 was isolated from the white cloud disease of *Micropterus salmoides*, which was dying. After the pure culture, the artificial infection was done to the health *Micropterus salmoides*, the ways of the artificial infection had three: abdominal cavity (0.3mL/nos), wound soaking, and soaking. The concentration of bacterial suspension was determined by McF and live bacterial counting technique. The control was substitute for physiological saline. The concentration of soaking was $2.31 \times 10^5$ Cells · $mL^{-1}$. The result showed that the strain of bacterial 01314-1 had powerful pathogenicity and the mortality of health fishes was 100 %. The symptom of the artificial infection fishes was similar to that of the natural diseased fish, and one strain of bacterial which was isolated from the slime, blood, liver, kidney of the artificial infection fishes was the same to bacterial 01314-1. According to Bergey's manual of systematic bacteriology and API 20NE system indentification, the pathogen of *Micropterus salmoides* was identified *Burkhol cepacid*. *Burkhol cepacid* often occurred in sea and oil, which caused many disease of human and animal. The main sign was septicaemia, infection and abscess.

Serum was made of the blood from diseased fishes and health fishes, which of the blood biochemical indices was determined by DADE BEHRING biochemical automatic instrument. Compared with healthy fishes, the blood biochemical indices showed that serum enzymes of ALT, AST, LDH and the number of total protein, urea nitrogen, creatinine, $K^+$ of diseased fishes all increased remarkably, but the contents of glucose, cholesterol, $Na^+$, $Cl^-$ decreased. The results indicated that the liver, kidney, and muscle of the fishes had diseased and many important physiological functions were seriously destroyed, after *micropterus salmoides* was infected. The increasing of total protein, urea nitrogen and creatinine in the serum showed that the fish body lost water and the blood concentrated, which was accord with the phenomenon of a lot of slime with the diseased fish.

The way of the drug sensitivity test was the same to the others, and the Chinese herbal medicine paper was reference to Xu-B's study on bacteriostasis drugs of pathogenic bacterium on shrimp. The results of drug sensitivity test showed that five chemotherapeutants (Compound sulfamethoxazole, Amikacin, Sulfamethoxazole, Tobramycin, Lincomycin) and three Chinese herbal medicines (Rhus, Armeniaca, Schisandra) were most effective against the pathogenic bacteria. So these drugs could prevent and treat the white cloud disease of *Mmicropterus salmoides*.

**Key words:** *Micropterus salmoides;* White cloud disease; *Burkhol cepacid;* Blood biochemical indices; Drug sensitivity test